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Identification and Target-modifications of Temporin-PE: A Novel Antimicrobial Peptide in the Defensive Skin Secretions of the Edible Frog, *Pelophylax* kl. *esculentus*

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Abstract

A potent natural antimicrobial peptide named temporin-PE was identified and encoded from the skin secretions of *Pelophylax* kl. *esculentus* via "shotgun" cloning and LC-MS/MS fragmentation analysis. Target-modifications were carried out to further enhance the antimicrobial and anti-proliferative bioactivities, whilst decreasing the hemolytic effect. A range of bioassays demonstrated that replacing a proline with a tyrosine residue resulted in a loss of the bioactivity against Gram-negative bacteria, but dramatically improved the hemolytic and anti-proliferative activity, indicating the FLP- motif influences the hemolytic activity of temporins. Moreover, the coupling of TAT to the peptide dramatically improved its antimicrobial activity, indicating coupling TAT to these peptides could be considered as a potential tool to improve their antimicrobial activity. Overall, we have shown that targeted modifications of this natural antimicrobial peptide can adjust its bioactivities to help its development as an antibiotic or anti-proliferative agent.

Abbreviations：AMP, antimicrobial peptide; GM, the geometric mean; TI, the therapeutic index

Keywords: temporin; peptide design; cytotoxicity; antimicrobial

Introduction

Anurans are a group of morphologically specialized amphibians widely distributed around the world. *P*. kl. *esculentus* is a common species of frog in Europe, where it is also known as the edible frog. Although *P*. kl. *esculentus*, previously known as "*Rana esculenta*", is a hybrid of the pool frog (*Rana lessonae*) and the marsh frog (*Rana ridibunda*), it possesses superior skin peptide immune defenses. Compared to the parental species, this hybrid species not only produces the most diverse peptides, but the antibiotic activity and stability of its antimicrobial peptides (AMPs) are also superior [1]. Therefore, the innate immune defense system of *P*. kl. *esculentus* is worthy of detailed research.

To date, more than thirty different AMPs have been discovered from the edible frog [2-9], most of which are brevinins [4, 6-8], esculentins [4] and temporins [5, 6, 9, 10]. Besides, there are also protease inhibitors [11] and bradykinins [12] in the skin secretions of *P*. kl. *esculentus*. According to the literature, temporins are a group of AMPs with potential to be developed as novel antibiotic agents due to their ability to kill microorganisms, including antibiotic-resistance bacteria. Temporins are short, C-terminally α-amidated, highly hydrophobic and weakly charged or non-charged [13]. Their secondary structures are usually linear and α-helical, which is one of the critical elements contributing to their cytotoxic effects upon microbial and cancer cells, due to their ability to destabilize membranes. Although, it is common for some AMPs to exhibit activity against cancer cells, and temporins have been shown to permeabilize several cell types, there are few reports about the impact of temporins on cancer cells, indeed, only the antimicrobial temporin-1CEa and temporin-La have been shown to exert significant activity against cancer cells [14-17]. The mechanism by which temporins impact upon cancer cells has been shown to relate to intracellular mechanisms involving mitochondria, including Ca^{2+} leakage, the collapse of mitochondrial membrane potential and over-generation of reactive oxygen species and membrane-denaturation due to the electrostatic interaction between the cationic peptides and anionic cell membrane [14, 17].

Materials and Methods

Specimen Biodata and Harvesting of Skin Secretion

The frogs were obtained, fed and skin secretions were harvested from the edible frog as described previously [9]. These procedures were subject to ethical approval and carried out under appropriate UK animal research personal and project licenses.

Molecular Cloning of Temporin-PE Precursor-Encoding cDNA

The "shotgun" cloning was carried out as previously described [9]. A degenerated primer (5'-GAWYYAYYHRAGCCYAAADATGTTCA-3') (W = A or T; Y = C or T; H = A or C or T; R = A or G; D = A or G or T) was subjected to 3'-RACE reaction, from which the products were cloned and subsequently sequenced.

Identification of Temporin-PE in the skin secretion

The skin secretion was fractionated by RP-HPLC and all fractions were subjected to MALDI-TOF mass spectrometer. Then the fraction containing the same molecular weight of the putative mature peptide was analyzed by MS/MS fragmentation sequencing. The experimental procedures for RP-HPLC, MALDI-TOF and LC-MS/MS have been described previously [18].

Design and Chemical Synthesis of Peptides

Here, the natural-occurring temporin-PE was employed as a template for the targeted design. The temporins present in *P*. kl. *esculentus* are structurally similar to peptides discovered in wasp venom, which display a conserved N-terminal FLP motif which caused hemolysis [19]. Therefore, to determine if this motif was causing a similar impact in temporin-PE, we modified the FLP motif to produce temporin-PEa and examined its hemolytic activity. Particularly, substituting Pro^3 by Tyr^3, designed by the genetic algorithm (GA) mutation in HeliQuest tool, was used to change the backbone of temporin-PE but remained the similar physicochemical parameters, namely temporin-PEa. Meanwhile, another analog was designed by coupling a TAT (-GRKKRRQRRR-) at the C-terminus of temporin-PE, namely temporin-PEb, in order to enhance the cell penetrating

activity as well as investigate the potential intracellular targets of temporin-PE [20]. All peptides were chemically-synthesized by solid phase Fmoc chemistry [18].

Predicted Physicochemical Parameters, and Secondary Structures of Peptides

The secondary structure predictions and models were processed using the I-TASSER server [21-24]. The helical wheels and the physiochemical parameters of peptides were predicted using the HeliQuest tool [25, 26]. The secondary structures of all peptides were examined by circular dichroism (CD) and analyzed by the K2D3 web server as described before [18].

Antimicrobial Assay

The minimal inhibitory concentrations (MICs) of peptides were evaluated by the standard microdilution assay described previously [18]. Six different microorganism strains, *Candida albicans* (NCPF 1467), *Staphylococcus aureus* (NCTC 10788), *Escherichia coli* (NCTC 10418), Methicillin-resistant *S. aureus* (MRSA) (NCTC 12493), *Enterococcus faecalis* (NCTC12697) and *Pseudomonas aeruginosa* (ATCC 27853), were utilized in this assay. The geometric mean (GM) of MICs for each peptide to inhibit the growth of *C. albicans*, *S. aureus*, *E. coli*, MRSA, *E. faecalis* and *P. aeruginosa*, was calculated to estimate the overall antimicrobial activity of peptides. The therapeutic index (TI) of the peptide was shown as the ratio of HC_{50} for erythrocytes to the GM. The HC_{50} here is the concentration of a peptide inducing 50% erythrocyte hemolysis.

Time-killing assay

The time killing assay was performed following the Corinne's protocol with modifications [27]. Briefly, *S. aureus* (NCTC 10788) and MRSA (NCTC 12493) were employed in this assay. 4μM, 2μM and 1μM of each peptide were applied for *S. aureus* and 8μM, 4μM and 2μM were applied for MRSA. Appropriate time points were selected, as indicated, to observe the viable counts.

Anti-proliferation assay

The anti-proliferation assay was performed using MTT as in our previous studies [28, 29]. The non-small cell lung cancer cell line NCI-H157, human neuronal glioblastoma cell line U251MG, human prostate carcinoma cell line PC-3, melanoma cell line MDA-MB-435s and human microvessel endothelial cells HMEC-1 were utilized in this assay. The GMs for anti-proliferative activity were calculated. The TI value of each peptide was shown as the ratio of IC_{50} for HMEC-1 cells to the GM for four types of cancer cells.

Hemolysis Assay

The hemolysis bioactivity of the three peptides against defibrinated horse erythrocytes was determined following the same protocol as used previously [11, 28]. 1% TritonX-100 was employed as the positive control.

Statistical Analysis

All the bioactivity assays had five independent replicates. Data were subjected to statistical analysis using Prism (Version 5.0, U.S.A). The one-way ANOVA was used to calculate the significance of the sample set. Error bars in the charts indicate standard error of the mean.

Results

Molecular cloning of A Novel Precursor-encoding cDNA

A nucleotide and translated amino acid sequence of an open-reading frame (ORF) encoding a potential antimicrobial peptide precursor were obtained via shotgun cloning (Figure 1). The petide consists of 62 amino acid residues, divided into five definite domains including a highly conserved putative signal peptide region containing 23 amino acid residues, an acidic amino acid residue-rich "spacer" region, a typical -Lys-Arg- propeptide convertase processing site, a putative mature peptide region containing 13 amino acid residues, namely temporin-PE, and a C-terminal -Gly-Lys- propeptide which acts as an amide donor to C-terminal amindation of the mature peptide. The nucleotide sequence of temporin-PE has been deposited to GenBank under the accession code MF957260.

Confirmation of the Primary Structure of Temporin-PE

Abundant peaks were fractionated from the skin secretions of *P*. kl. *esculentus* by RP-HPLC (Figure 2A). MALDI-TOF confirmed the peak at 152 min was the fraction containing the predicted mature peptide (Figure 2C) and this this fraction was further analyzed via MS/MS fragmentation analysis (Figure 2B and Table 1).

Secondary Structures and Physicochemical Properties of Temporin-PE, Temporin-PEa and Temporin-PEb

Temporin-PE, Temporin-PEa and Temporin-PEb were all predicted to be typical α-helical peptides (Figure 3A-C). The predicted helical wheel structures of these peptides demonstrated that all three contained a hydrophobic face, exhibiting that higher amphipathy of temporin-PEb than which of the other two (Figure 3D-F). Temporin-PE and temporin-PEa displayed similar physicochemical properties, while temporin-PEb exhibited a larger α-helical domain, higher net positive charges and hydrophilicity (Table 2). CD spectra of the peptides confirmed that the peptides were random coil but formed α-helical structures in the membrane-mimic environment (Figure 3G&H). Although the actual contents of α-helical domains of each peptide are lower than the predicted ones, it reveals that the temporin-PEb possesses the largest α-helical domain, whereas temporin-PEa contained the smallest.

Antimicrobial assay

Temporin-PE potently inhibited the growth of the pathogenic fungi (*C. albicans*), the Gram-positive bacteria *S. aureus* and MRSA, but showed lower efficacy against the Gram-negative bacteria such as *E. faecalis* and *P. aeruginosa.* Temporin-PEa exerted similar bioactivity except against the Gram-negative bacteria, *E. coli* and *P. aeruginosa* (Table 3). However, temporin-PEb showed the most potent antimicrobial bioactivity against all six microorganisms.

Time-killing kinetics assay

Temporin-PE and temporin-PEb showed concentration-dependent and time-dependent bactericidal activity against *S. aureus* and MRSA, however, temporin-PEa did not impact upon these two strains at all tested concentrations (Figure 4A-F). Temporin-PEb was the most potent AMP starting to kill *S. aureus* at concentrations from 1μM, while taking 30 minutes to kill MRSA at concentrations from 4μM. Temporin-PE displayed potent activity against *S. aureus* at 8μM, but was much less potent against MRSA.

Cancer cell viability assay

Since temporin-PE and the two designed analogs exerted cytotoxicity to pathogenic microorganisms and erythrocytes, the ability of them functioning to cancer cells were also studied. The result indicated that two designed peptides possessed more efficient anti-proliferative activity than temporin-PE (Figure 4G-K). However, unlike temporin-PEa without seriously cytotoxicity on normal human microvessel endothelial cells HMEC-1 at the 10μM, temporin-PEb showed nonspecific cytotoxicity towards all tested cell lines. Temporin-PEa displayed the relatively highest TI values than the other peptides (Table 4).

Hemolysis assay

Temporin-PE, temporin-PEa and temporin-PEb exhibited different degrees of hemolysis on horse erythrocytes with HC_{50} of 39.64μM, 531.7μM and 44.64μM, respectively. Temporin-PEa and temporin-PEb possessed lower hemolytic activity than temporin-PE, especially temporin-PEa, with 13-fold less hemolytic activity.

Discussion

To date, except temporin-PE, only five temporins have been isolated from *P*. kl. *esculentus* [5, 6, 9, 10]. According to the literature, temporin-PE possesses potent antimicrobial activity which is 25-fold, 4-fold and 2.5-fold stronger than the best inhibitory effects of other reported temporins in *P*. kl. *esculentus* against *C. albicans*, *S. aureus* and *E. coli* respectively (Table 5). It is noteworthy that most temporin members have the common motif FLP- at their N-termini and a leucine at the

second position near their C-termini, as well approximately 70% hydrophobic residues, which make them highly hydrophobic [30]. Compared to temporin-1Ec and temporin-1Ee, temporin-PE exhibited a more potent antimicrobial activity, something which could be explained by the position of the positive charge in its sequence. Temporin-PE, containing a lysine residue at position 7 (Lys^7), possesses a more potent antimicrobial effect, especially against the growth of fungi, as Lys^7 is located on the hydrophilic face of the helix domain, while a lysine residue at position 11 might not be on the hydrophilic face unless the helical domain extended to the C-terminus [31]. We therefore presumed that the lysine located on the hydrophobic region or random domain could not play roles as well as that on the hydrophilic region of temporins especially in the field of electrostatic interactions and bindings with negatively charged residues in the cell membranes.

Temporin-PEa retained its antimicrobial activity except which was against Gram-negative bacterium. Both peptides share the similar secondary structure and physicochemical properties relating to antimicrobial activity, which suggested the significance of Pro on membrane interaction between peptides and Gram-negative bacterium membrane. Usually, AMPs firstly bind lipopolysaccharide (LPS) micelles when they interact with Gram-negative bacterium. Such binding might lead some AMPs to translocate through the outer membrane of Gram-negative bacterium, as the magnesium and calcium ions that hold together LPS would replace metal ions, breaking the outer membrane, and resulting in facilitating entry of additional AMPs from the exterior [32]. Previous studies have shown that proline plays an essential role in outer membrane disruption and bacterial cell lysis activity, as the more proline residues present, the stronger the binding between AMPs and LPS [33]. Therefore, removing the proline might decrease the LPS binding affinity, meaning temporin-PEa could not overcome the LPS hurdle and the integrity of these bacterial cells would not be disrupted, resulting in a loss of activity against Gram-negative bacterium. Moreover, compared to tyrosine, proline, which usually forms a slight hinge structure, is more possible to make peptides more easily to fasten on the zwitterionic membrane. That is confirmed what we had anticipated, the temporin-PEa showed a vastly reduced hemolytic activity indicating this FLP motif is again important in these peptides. However, it increased the anti-proliferative activity against cancer cells. As it is reported, the plasma membrane surface of

cancer cells contains abundant phosphatidylserines, glycoproteins, gangliosides and some other anionic molecules, some of which are often over-expressed in cancer cell lines [34]. That is presumably the reason why the substitution of proline with tyrosine leads to a decreased toxicity towards normal cells, but shows increased anti-proliferative activity toward cancer cells. We would therefore hypothesize this anti-proliferative activity results from the phenolic hydroxyl moieties binding to target molecules, such as sialic acids of the O-glycoproteins, N-glycoproteins and gangliosides, which are overexpressed on cancer cells.

TAT is an arginine-rich decapeptide derived from HIV-1, an ideal cell-penetration segment to transit drugs through cell membranes via membrane translocation and endocytosis, with no hemolytic effects, mild antibiotic activity and inhibits the growth of fungi with MICs ranging from 3μM to 24μM [35]. We hypothesized that the electrostatic forces between temporin-PEb and anionic cell membranes would become much stronger when eight positive charges were introduced as part of TAT. Compared to temporin-PE, temporin-PEb demonstrated an 8-fold and 64-fold stronger antimicrobial effect against *E. coli* and *P. aeruginosa* respectively, 4-fold stronger against *C. albicans* and *E. faecalis*, and two-fold against *S. aureus* and MRSA. In addition, temporin-PEb also acted more rapidly against these microporganisms. Although the antimicrobial mechanism of temporin-PEb was not verified experimentally, it is presumed that the addition of TAT improved the electrostatic interaction, as well as helping the peptide to penetrate into the cytoplasm to interfere the biological processes such as DNA replication and protein synthesis, leading to its markedly improved antimicrobial activity [36]. Meanwhile, the anti-proliferative activity of temporin-PEb was also increased against all the cell lines examined. So we hypothesize that temporin-PEb might not only disrupt the plasma membrane through the permeabilization, but also involve the intracellular targets, such as mitochondrial membrane, after the penetrating the cell membrane by TAT.

In summary, this study has identified a novel temporin which shows potent antimicrobial activity. We then designed target-modifications to enhance the antimicrobial and anti-proliferative bioactivities of this peptide, whilst decreasing its hemolytic effect. The substitutions made demonstrated that the FLP motif, as previously shown for other similar peptides, is associated with the hemolytic activity of temporin-PE. In addition, modifying the FLP motif also decreased the

potency of this peptide against Gram-negative bacteria, while enhancing its anti-proliferative activity. Furthermore, this work shows that introducing a TAT peptide at the C-terminus of temporin dramatically increased the antimicrobial activity of temporin-PE. Therefore, this study has opened up new perspectives on how modifying natural AMPs can alter their bioactivities.

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Legends to Figures and Tables

Figure 1. Nucleotide and translated ORF amino acid sequences of the precursor cDNA encoding the peptide temporin-PE, from the skin secretions of *P*. kl. *esculentus*. The putative signal peptide sequence is single-underlined, the mature peptide sequence is double-underlined, and an asterisk indicates the stop codon.

Figure 2. (A) RP-HPLC chromatogram of skin secretion of RP-HPLC *P*. kl. *esculentus* at a wavelength of 214nm. The peak with an arrow is the peak of temporin-PE. (B) The annotated MS/MS spectrum of the RP-HPLC fraction of temporin-PE. (C) MALDI-TOF mass spectrum of the RP-HPLC fraction containing temporin-PE.

Figure 3. Predicted secondary structure models of (A) temporin-PE (B) temporin-PEa, (C) temporin-PEb and helical wheel projections of (D) temporin-PE, (E) temporin-PEa, (F) temporin-PEb; CD spectra of temporin-PE and its analogs (50μM) (G) in 10 mM ammonium acetate water solution and (H) in 50% TFE/10 mM ammonium acetate water solution. The arrows indicate the hydrophobic regions.

Figure 4. The time-killing kinetics of three peptides at different concentrations of (A) 4μM, (B) 2μM, (C) 1μM against *S. aureus* and (D) 8μM, (E) 4μM, (F) 2μM against MRSA; The cell viabilities of (G) NCI-H157 (H) U251MG (I) PC-3 (J) MDA-MB-435s (K) HMEC-1 and the hemolytic effects (L) after treatment of temporin-PE and its analogs

Table 1. Expected b- and y-ions arising from collision induced of the doubly-charged precursor ion. Actual fragment ions observed in MS/MS fragmentation are marked with the single-underlined bold typeface.

Table 2. Primary structures, predicted secondary structures and physicochemical properties of temporin-PE, temporin-PEa and temporin-PEb

Table 3. MICs, GMs and TI values of Temporin-PE, Temporin-PEa and Temporin-PEb against six microorganisms

Table 4. Impact of temporin-PE, temporin-PEa and temporin-PEb on cancer cell viability

Table 5. A comparison of the primary structures and MICs of temporins discovered in *P.* kl. *esculentus*

Figure 1.

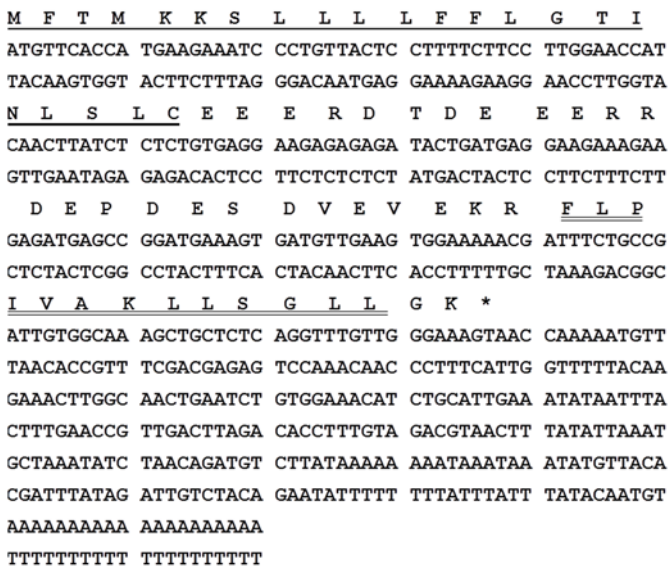
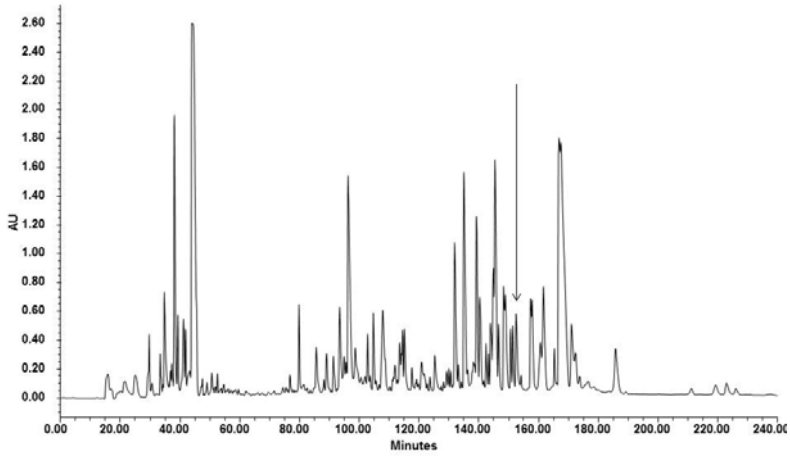
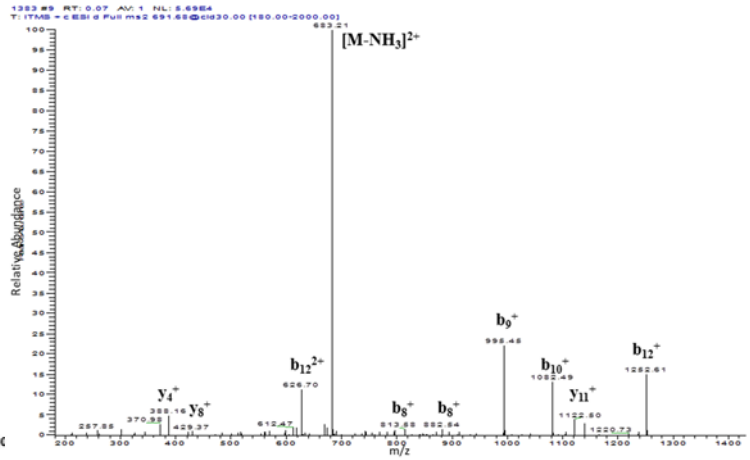
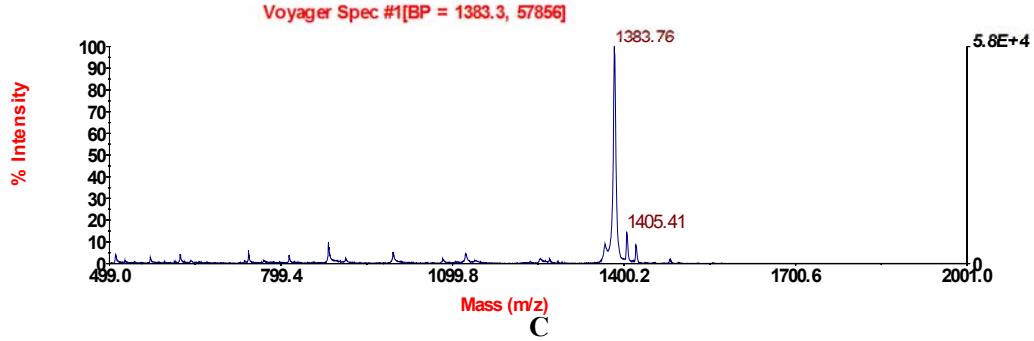

M F T M K K S L L L F F L G T I
1 ATGTTCACCA TGAAGAAATC CCTGTTACTC CTTTTCTTCC TTGGAACCAT
TACAAGTGGT ACTTCTTTAG GGACAATGAG GAAAAGAAGG AACCTTGGTA
N L S L C E E E R D T D E E E R R
51 CAACTTATCT CTCTGTGAGG AAGAGAGAGA TACTGATGAG GAAGAAAGAA
GTTGAATAGA GAGACACTCC TTCTCTCTCT ATGACTACTC CTTCTTTCTT
D E P D E S D V E V E K R F L P
101 GAGATGAGCC GGATGAAAGT GATGTTGAAG TGGAAAAACG ATTTCTGCCG
CTCTACTCGG CCTACTTTCA CTACAACTTC ACCTTTTTGC TAAAGACGGC
I V A K L L S G L L G K *
151 ATTGTGGCAA AGCTGCTCTC AGGTTTGTTG GGAAAGTAAC CAAAAATGTT
TAACACCGTT TCGACGAGAG TCCAAACAAC CCTTTCATTG GTTTTTACAA
201 GAAACTTGGC AACTGAATCT GTGGAAACAT CTGCATTGAA ATATAATTTA
CTTTGAACCG TTGACTTAGA CACCTTTGTA GACGTAACTT TATATTAAAT
251 GCTAAATATC TAACAGATGT CTTATAAAAA AAATAAATAA ATATGTTACA
CGATTTATAG ATTGTCTACA GAATATTTTT TTTATTTATT TATACAATGT
301 AAAAAAAAAA AAAAAAAAAA
TTTTTTTTTT TTTTTTTTTT

Figure 2.


AU
Minutes
0.00
20.00
40.00
60.00
80.00
100.00
120.00
140.00
160.00
180.00
200.00
220.00
240.00


[M-NH3]2+
b9+
b12+
b12 2+
b10+
y11+
y4+
y8+
b8+
b8+
m/z

A **B**


Voyager Spec #1[BP = 1383.3, 57856]
% Intensity
1383.76
1405.41
5.8E+4
Mass (m/z)
499.0
799.4
1099.8
1400.2
1700.6
2001.0

C

Figure 3.

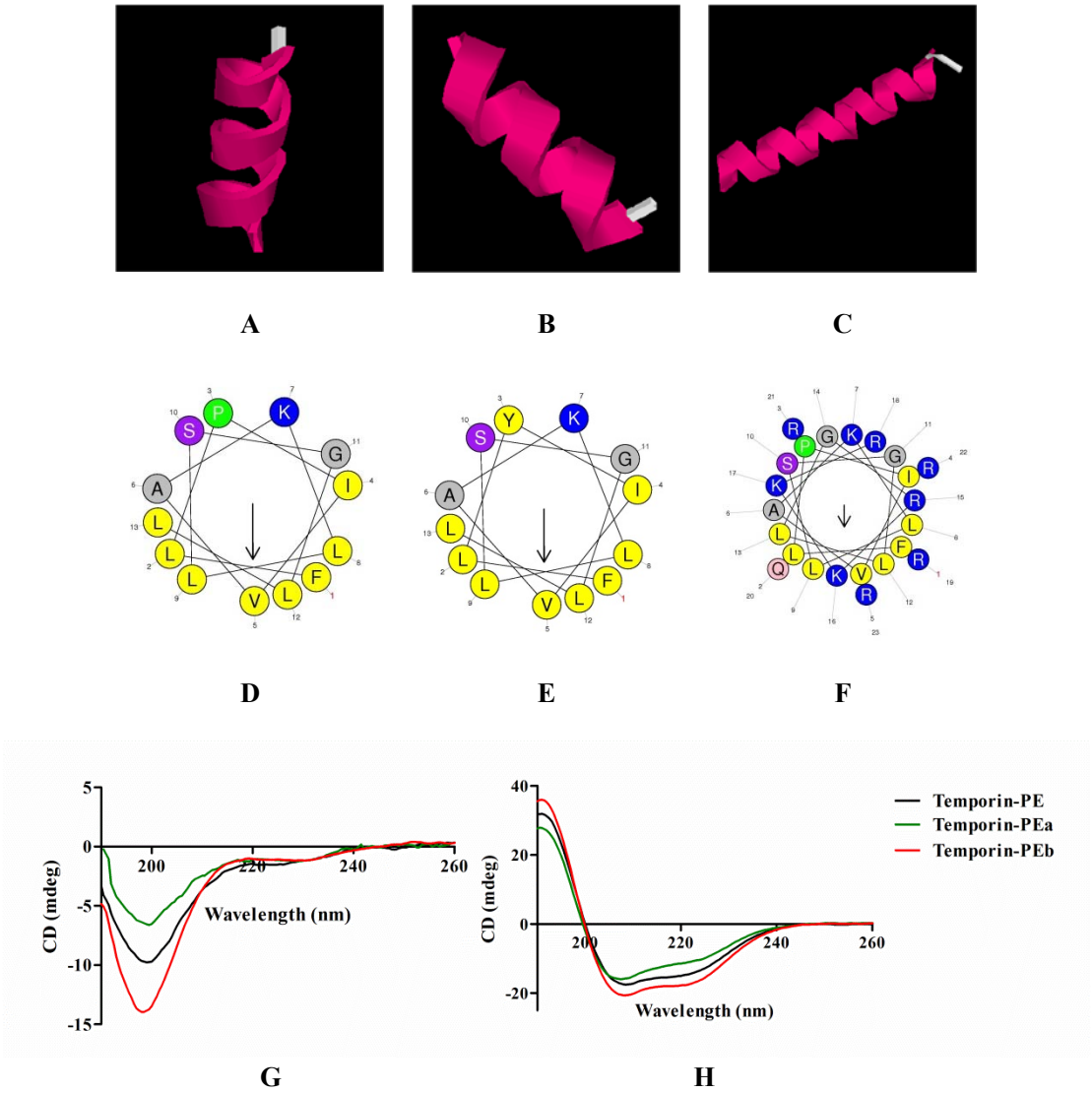

A
B
C
D
E
F
G
H
CD (mdeg)
Wavelength (nm)
Temporin-PE
Temporin-PEa
Temporin-PEb

Figure 4.

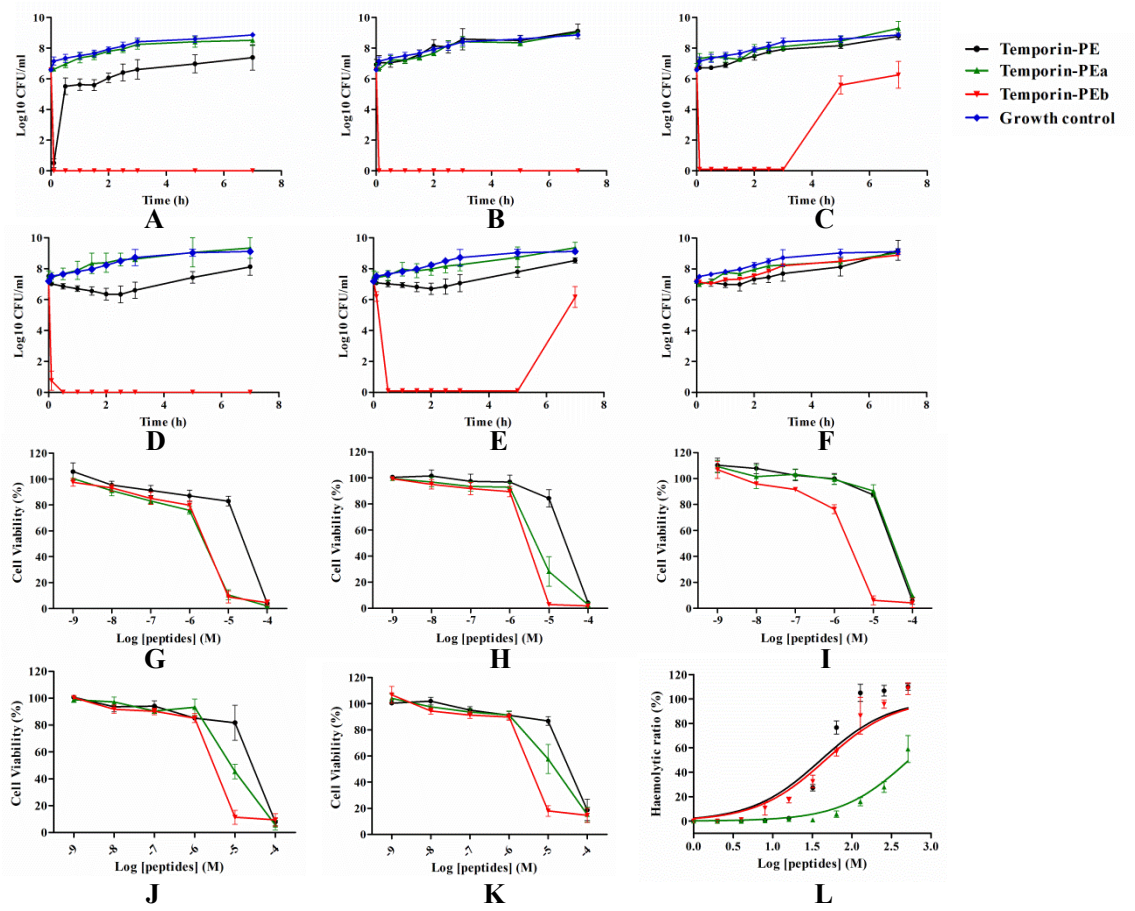

Temporin-PE
Temporin-PEa
Temporin-PEb
Growth control
Log10 CFU/ml
Time (h)
A
B
C
D
E
F
Cell Viability (%)
Log [peptides] (M)
G
H
I
J
K
Haemolytic ratio (%)
L

Table 1.

#1	b(1+)	b(2+)	Seq.	y(1+)	y(2+)	#2
1	148.0757	74.54149	F			13
2	**261.15977**	131.08352	L	**1235.84503**	**618.42615**	12
3	358.21254	179.60991	P	**1122.76096**	**561.88412**	11
4	471.29661	236.15194	I	**1025.70819**	**513.35773**	10
5	**570.36503**	285.68615	V	**912.62412**	456.8157	9
6	**641.40215**	321.20471	A	**813.5557**	407.28149	8
7	**769.49712**	385.2522	K	**742.51858**	**371.76293**	7
8	**882.58119**	441.79423	L	614.42361	307.71544	6
9	**995.66526**	498.33627	L	**501.33954**	251.17341	5
10	**1082.69729**	541.85228	S	**388.25547**	194.63137	4
11	**1139.71876**	**570.36302**	G	**301.22344**	151.11536	3
12	**1252.80283**	**626.90505**	L	244.20197	122.60462	2
13			L-Amidated	131.1179	66.06259	1

Table 2.

	Peptides		
	Temporin-PE	**Temporin-PEa**	**Temporin-PEb**
Sequence	FLPIVAKLLSGLL$_a$	FL**Y**IVAKLLSGLL$_a$	FLPIVAKLLSGLL**GRKKRRQRRR**$_a$
Structure Prediction[1]	CCHHHHHHHHCCC	CCHHHHHHHHHCCC	CCHHHHHHHHHHHHHHHHHHHHCC
Confident Score	9127999975329	9613998872439	91389999998861068877329
Actual α-helix (%)	47.65	40.37	55.43
Nonpolar residues (%)	76.92	76.92	43.48
Hydrophobicity (H)	1.024	1.042	0.220
Hydrophobic moment (μH)	0.570	0.552	0.304
Net charge	+2	+2	+10

[1] C, Coil; H, Helix

Table 3.

Microorganism / Peptide	MIC (μM)						GM (μM)	TI
	C. albicans	***S. aureus***	***E. coli***	**MRSA**	***E. faecalis***	***P. aeruginosa***		
temporin-PE	4	2	16	4	8	128	8.98	4.41
temporin-PEa	2	1	>512	2	2	>512	10.08	52.23
temporin-PEb	1	1	2	2	2	2	1.59	28.08

Table 4.

	Cell line	Peptide		
		temporin-PE	**temporin-PEa**	**temporin-PEb**
IC_{50} (μM)	NCI-H157	34.56	3.398	3.464
	U251MG	25.13	3.520	3.087
	PC-3	38.56	27.62	3.051
	MDA-MB-435s	33.23	9.864	3.483
	HMEC-1	58.62	40.30	9.976
GM (μM)		32.48	7.555	3.265
TI		1.805	5.334	3.055

* NC, not calculated

Table 5.

Peptide	sequence	Hydrophobicity	Hydrophobic	Net	MIC (μM)

		(H)	moment (μH)	charge	*C. albicans*	*S. aureus*	*E. coli*
temporin-PE	FLPIVAKLLSGLL$_a$	1.024	0.570	+2	4	2	16
temporin-1Ec	FLPVIAGLLSKLF$_a$	1.031	0.598	+2	>160	10	40
temporin-1Ee	FLPVIAGVLSKLF$_a$	0.994	0.586	+2	>100	8	>100
temporin-1Re	FLPGLLA---GLL$_a$	1.132	0.507	+1	>160	60	>160
A1	FLPAIAGILSQLF$_a$	1.028	0.587	+1	ND[1]	>200	>50
B9	FLPLIAGLLGKLF$_a$	1.071	0.585	+2	ND	ND	ND

[1] ND, not determined